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Microbiological safety assessment of two types of locally made cheese sold in Malete, Kwara State

Adedayo Majekodunmi Racheal, Shittu Bushola Rukayya*, Hammed Bukola C

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Author Affiliation:
Department of Microbiology, Faculty of Pure and Applied Sciences, Kwara State University, Malete, Nigeria

***Corresponding author**
Department of Microbiology, Faculty of Pure and Applied Sciences, Kwara State University, Malete
Nigeria
Email: shitturuka@gmail.com

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ABSTRACT

Cheese is a protein rich food with high nutritional benefits. Fulani cheese is a dairy product derived from milk that is produced in a wide range of flavors, textures and forms by coagulation of the milk protein while soy cheese is derived from the coagulation of soya beans milk. The study assesses the microbiological safety of Fulani and soy cheese sold in Malete, Kwara State. The cheese samples were purchased from local sellers in Malete village. Microbial isolation was done through serial dilution and pour plate technique; colonies obtained were identified morphologically and biochemically. The identified bacterial isolates were *Salmonella*, *Enterobacter*, *Serratia*, *Klebsiella*, *Citrobacter*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* and *Proteus* sp while the fungal isolates were *Penicillium*, *Rhodotorula*, *Aspergillus* and *Fusarium* spp. *Staphylococcus aureus* had the highest percentage of occurrence in both cheese samples, followed by *Salmonella* and *Klebsiella* species. Most of the bacterial isolates are human pathogenic organisms. The fungal isolated are known food spoilage agents with potential of releasing mycotoxins in food. It was concluded that both Fulani and Soy bean cheese sold in Malete village are contaminated heavily and could pose health threat to consumer.

Keywords: Fulani cheese, Soy-bean cheese, Bacteria, Fungi, Pathogen

1. INTRODUCTION

In recent years, small-scale and cottage industries have evolved in cheese production worldwide and Nigeria is no exception (Costanzo et al., 2020). This can be traceable to the increase in demand for ready to eat foods of which local cheese is prominent (Muhammad et al., 2016). The cost of procuring animal protein like meat and fish is almost beyond the reach of the majority in developing countries who often find alternatives in locally produced cheese (Bristone et al., 2018), moreover the love for "quick food" among the students and working class. Food-safety hazard generally refers to the presence of any contaminants in a food which may be biological, chemical or physical agent that could cause adverse health consequences for consumers (McDonald and Scott,

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2018; Costanzo et al., 2020; Owusu-Kwarteng et al., 2020). Such hazards may be introduced into the food at any time during processing.

Consuming microbiologically contaminated food often results in ill health of various status and sometimes death (Bristone et al., 2018; Costanzo et al., 2020). Food contamination has been recognized as a global challenge in several documents and reports (WHO, 2015; Fakuda, 2015; WHO, 2020). An estimated 600 million – almost 1 in 10 people in the world – fall ill after eating contaminated food and 420, 000 die every year, resulting in the loss of 33 million healthy life years (DALYs) (WHO, 2020). Microbial food poisoning/illnesses occurs as a result of eating food or water contaminated by microorganisms (bacteria, mould, virus, parasites) or their toxic metabolites or both. The symptoms of food poisoning vary and most often depends on the causative agent and most often include but not limited to abdominal cramps, nausea, vomiting, diarrhea, and fever (Owusu-Kwarteng et al., 2020; WHO, 2020).

Dairy cheese is mostly milk curd - substance formed from the coagulation of milk, pressed or molded into a solid mass (LaBarbera, 2012). The major content of cheese is concentrated milk solids, water, rennet, salt and sometimes bacterial cultures and calcium chloride (Farah and Fischer, 2004). Cheese can also be prepared from non-dairy products such as soybean (Schaeffer, 2012; Bristone et al., 2018). Soybean cheese is a healthy, rich and less expensive source of nutrient especially for the developing countries (Tamime et al., 2011; Nazim et al., 2013; Bristone et al., 2018). Soybean cake is made from a legume and legumes are known to be very rich sources of protein. It is the most important and popular food product from soybean in many communities also gaining increasing popularity (Yusuf and Ali, 2013; Bristone et al., 2018).

Soybean cheese was developed some 2000 years ago and has become the world's most popular soy food product due to its high protein content (Egbo, 2012) and to its high nutritional and excellent functional properties. It is very rich in carbohydrates and oil, antioxidants, essential nutrients including many amino acids and well digested if properly processed (Beatrice and Oguntoyinbo, 2012; Kolawole et al., 2015). Soy-bean cheese has been accepted as meat substitute in rural and urban areas of Nigeria. Local processing of soy-cheese is usually done at the home level and usually has no good processing methods (Bristone et al., 2018).

The high protein content and other nutritional content of these products coupled with high moisture content makes it suitable to the growth of microorganisms, especially with no good manufacturing process, poor hygiene of handlers and environment with poor storage and vending conditions. The product though very nutritious is usually of poor shelf life as describe by Fasoyiro, (2011). It is important to provide current knowledge on microbial contaminants and possible health hazards associated with local cheese consumption in Malete, with student community of about 10,000 in population and the location of Kwara State University, this study aims to investigate the microbiological safety of two locally produced, vended and consumed cheese: Fulani cheese and soya beans cheese in Malete.

2. MATERIALS AND METHODS

Collection of Samples

Fresh samples of both the Fulani and soyabean cheese were obtained from Malete village in clean sterile containers and were conveyed to the laboratory immediately for analysis.

Isolation of Microorganisms

Serial dilution was carried out on the samples, plating was done using 0.1 ml of the homogenate inoculated into Petri dishes containing nutrient agar (for general bacteria), Saboraud dextrose (for fungi), Mannitol salt (for *Staphylococcus aureus*), Eosin Methylene blue (for faecal coliform) and MacConkey agar (for total coliform) via pour plate technique. The plates were swirl round for proper mixing before the content was allowed to solidify. The inoculated plates except Sabouraud dextrose agar (SDA) plates were incubated at 37^0C (44^0C for faecal coliform) for 24 hours while the Sabouraud dextrose agar (SDA) plates were incubated on the lab bench for 48 hours. The developed colonies of discrete organisms on plates were observed and counted (Oyeleke and Manga, 2008). The isolates were sub cultured repeatedly to obtain pure culture. The colonies from Fulani cheese were labelled as Fcwhile those from soy cheese were labelled as Sc for the soy bean cheese. The isolates were maintained on slants at 4^0C for further research.

Morphological and Biochemical Characteristics of isolates

Isolates were characterized morphologically and biochemically; the observable characters were used for identifying the organisms by comparing with standard reference organisms (Muhammad et al., 2016). Gram's reaction was done as described by them. Gram

positive cells will stain purple to blue while Gram negative cells stain red to pink. Biochemical tests done were: Catalase test, coagulase test, indole, citrate and oxidase tests and spore detection (Oyeleke and Manga, 2008).

Identification of Fungi

The fungal isolates were characterized and identified macroscopically and microscopically using morphological features like hyphal and spore shape, color, texture and other relevant features of the colonies. Staining with a drop of cotton blue in Lactophenol reagent was used to enhance microscopy. The characters obtained were compared with literature for identification using fungal atlas (Hunter and Bamett, 2000).

3. RESULTS

Total Microbial Counts

The total bacterial counts of Fulani cheese and soy beans are: - Bacteria on nutrient agar 28 and 25 x 10^3, total coliform are 25 and 29 x 10^3, *Staphylococcus aureus* 10 and 5 x 10^3, faecal coliform 22 and 2 x 10^3 cfu/ml respectively while the fungal count of Fulani and Soy bean cheese were 19 and 21 x 10^3 cfu/ml. These are shown in Table 1.

Table 1 Total Bacterial and Fungal Count

	Total Count x 10^3 cfu/ml				
Samples	Bacteria	*Staphylococcus aureus*	Faecal coliform	Total coliform	Fungi
Fulani cheese	28	10	22	25	19
Soy bean cheese	25	5	2	29	21

Percentage of Occurrence of Microbial Isolates from Fulani and Soy Bean Cheese

The percentage of occurrence of bacterial and fungal isolates from Fulani and Soy Bean Cheese are presented in Tables 2 – 4.

Table 2 Percentage of Occurrence of Bacterial Isolates from Fulani cheese

Identified organisms	Occurrence of identified organisms	% of occurrence of identified organisms
Salmonella species	2	15.38
Enterobacter species	1	7.69
Serratia species	1	7.69
Klebsiella species	2	15.4
Citrobacter species	1	7.69
Staphylococcus species	3	23.07
Escherichia coli	1	7.69
Pseudomonas species	1	7.69
Proteus species	1	7.69
Total	13	100

Table 3 Percentage of Occurrence of Bacterial Isolates from Soy Bean Cheese

Identified organisms	Occurrence of organisms	% of occurrence of identified organisms
Salmonella species	1	20
Staphylococcus species	2	40
Klebsiella species	1	20
Salmonella species	1	20
Total	5	100

Characteristics and Identification of Bacteria Isolates

Based on morphological and biochemical characteristics; these organisms *Salmonella* sp., *Enterobacter* sp., *Serratia* sp., *Klebsiella* sp., *Citrobacter* sp., *Staphylococcus* sp., *E. coli*, *Pseudomonas* sp. and *Proteus* species were identified. Result is presented in Table 5.

Table 4 Percentage of Occurrence of Fungal Isolates from Cheese samples

Identified Organisms	Occurrence of organisms	% of Occurrence of Identified Organisms
Penicillium species	1	25
Rhodotorula species	1	25
Aspergillus species	1	25
Fusarium species	1	25
Total	4	100

Table 5 Biochemical and Morphological Characteristics of Bacteria Isolates from Fulani and Soy Bean Cheese

Isolates	Oxidase	Citrate	Coagulase	Catalase	Indole	Gram's staining	Spore Staining	Probable organisms
Fc1N	-	+	-	+	-	-	-	*Salmonella* species
Fc2N	-	+	+	+	-	-	-	*Enterobacter* species
Fc3N	+	+	-	+	-	-	-	*Serratia* species
Fc4N	-	+	+	-	+	-	-	*Klebsiella* species
Fc5N	-	+	-	+	+	-	-	*Citrobacter* species
Sc1N	-	+	-	+	-	-	-	*Salmonella* species
Sc1MS	-	+	-	+	+	+	-	*Staphylococcus* species
Sc2MS	-	+	+	+	-	+	-	*Staphylococcus aureus*
Fc1MS	-	+	-	+	-	+	-	*Staphylococcus* species
Fc2MS	-	+	+	+	+	+	-	*Staphylococcus aureus*
Fc3MS	-	+	-	+	-	+	-	*Staphylococcus* species
Fc1EMB	-	+	-	+	-	-	-	*Klebsiella* species
Fc3EMB	-	-	-	+	+	-	-	*Escherichia coli*
Fc4EMB	-	+	-	+	+	-	-	*Pseudomonas* species
Sc1EMB	-	+	-	+	-	-	-	*Klebsiella* species
Fc1MA	+	+	-	+	+	-	-	*Proteus* species
Fc2MA	+	+	+	+	+	+	-	*Salmonella* species
Sc1MA	+	+	+	+	+	+	-	*Salmonella* species

Fc: Fulani cheese, Sc: Soya beans cheese, N: Nutrient Agar, MS: Mannitol Salt Agar, EMB: Eosin methylene blue, MA: MacConkey Agar

Morphological and Microscopical Characteristics of Fungal Isolates

Four species of fungal isolates were identified in both the Fulani and Soy bean cheese as members of the genera Penicillium, Rhodotorula, Aspergillus and Fusarium. The result is presented in Table 6.

Table 6 Morphological and Microscopical Characteristics of Fungal isolates

Isolates	Morphology characteristics	Microscopic observation	Organisms Isolated
Fc_1	Greenish at the center and whitish at the edge.	It has a brush-like structures with a clusters of flask shapes, the conidia are round, unicellular unbranching chain at the tips of the phialides.	*Penicillium* species
Fc_2	Appear pinkish on the plate due to presence of carotenoid pigments.	It is a round cells or oval cells and pseudohyphae are rarely present.	*Rhodotorula* species
Sc_1	Conidial are usually whitish growth on the plate	It has radiating conidial heads and the conidiophores appear rough	*Aspergillus* species
Sc_2	White mycelium growth on the plate	Some of the microconidia are curved while some were straight	*Fusarium* species

4. DISCUSSION

The cheese samples were found to be heavily contaminated with bacteria and fungi as presented in Table 1. The most prevalent organisms isolated in the present study include *Staphylococcus aureus*, *Salmonella* species and *Klebsiella* species. The isolation of similar pathogens has also been reported by previous workers from various foods raw and ready-to-eat foods including cheese Elfaki and Elhakim, 2011; Ameko et al., 2012; Muhammad et al., 2016). Most of the isolates were members of genera that are pathogenic therefore are of medical importance. Cheese has been reported to be one of the high-risk foods (Ogbolu et al., 2014). The nutritional status and the moisture content of cheese of any type support the growth of many types of microorganisms. Their occurrence in food however could pose various level of danger to the health of their consumers (Muhammad et al., 2016).

The percentage occurrence of *Salmonella* and *Staphylococcus* were high in both cheese samples; beyond allowable limits of organisms in ready to eat foods. Most of the bacterial isolated are enteric in nature though *Staphylococcus aureus* is a commensal and a normal human microbiota (Tables 2 and 3); the source of these microbial isolates could be attributed to lack of the practice of "Good Processing Skill" hygiene standard of the handlers and their environments. This is observation is in line with the work of (Ogbolu et al., 2014; Bristone et al., 2018) who reported heavy microbial contaminants in ready to eat foods. All the bacteria species encountered in these cheese samples are mostly members of organisms regarded as food borne pathogens. Their presence in food and consumption could result in ill health as a result of food infection and intoxication with different kind of symptoms (Owusu-Kwarteng et al., 2020). In severe cases; food poisoning could result in death Willey et al., 2011; WHO, 2020). The presence of proteolytic bacteria like *Pseudomonas, Serratia, Proteus* and *Staphylococcus* species in food can bring about food deterioration and poor shelf life essentially when the food is held at ambient temperature; the cheese tends to lose color, taste, odor and texture. Consumption of food infected with *Staphylococcus aureus* can lead to different types of Staph poisoning.

The presence of organisms of enteric origin is an indication of contamination of the samples with faecal materials. Species of other isolates are widely distributed in air, soil and water (Bristone et al., 2018). Their presence in the cheese samples might have occurred through poor handling by the food handlers/producers and consumers; who will normally touch the food with bare hand before making a purchase. Holding temperature, utensils, container for displaying the cheese and possibly packaging material could also aid the introduction and multiplication of microorganisms in the cheese samples (Adetunji et al., 2008; Ibrahim and Falegan, 2013). Enteric bacteria like *E. coli* and *Salmonella* species are responsible for various types of gastroenteritis and enteric fever. They are majorly responsible for food and water borne diseases (Muhammad et al., 2016; Bristone et al., 2018; WHO, 2020).

The fungal species encountered in both cheese samples are species of genera commonly known to be air borne. Such organisms have been earlier reported as food contaminants and spoilage agents of food and producers of mycotoxin in food essentially *Aspergillus* and *Fusarium.* This finding is in agreement with the work of Tauxe, (2002). *Penicillium* species**,** *Rhodotorula* species, *Aspergillus* species and *Fusarium* species generally produce spores in large number and when these spores get into suitable substates, they easily develop into the vegetative phase. The fungal contaminants could come from the environment and or the food handlers themselves or their utensils. The presence of fungal species in food results more often in food spoilage than food poisoning. When such spoilt foods are however consumed, it could result in food poisoning (Rawat, 2015; Saleh and Al-Thani, 2019).

The production of toxic metabolite by these fungal isolates in food can result in fungal food poisoning though fungal food poisoning is not usually as severe as bacterial food poisoning (Rawat, 2015; Saleh and Al-Thani, 2019). This study has demonstrated that most of the cheese consumed in Malete are contaminated with pathogenic bacteria and food spoilage fungi with potential of toxin production and do not meet the required quality and safety levels. Thus, could pose a health threat to the consumers and efforts to reduce level of contamination of this food are highly recommended because it is not only student from the university that are at risk, but also some individuals from the community.

5. CONCLUSION

Most cheese present in the market are not fit for direct consumption, both Fulani and soya bean cheese need to be further processed thoroughly to reduce the microorganisms present in them and make them fit for consumption. This calls for proper education of food handlers on good manufacturing processes, holding temperature, time lapse between production and dispensing of food and food packaging. Food safety measures put in place and strictly follow to enhance compliance will result in production and consumption of microbiologically safe foods and consequently fewer trips to hospital.

Informed consent

Not applicable.

Ethical approval

Not applicable. Food safety measures are followed.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Adetunji VO, Alonge DO, Singh RK, Chen J. Production of wara, a west African soft cheese using lemon juice as a coagulant. J Food Sci Tech 2007; 5-7. doi: 10.1016/j.lwt.2007.02.012
2. Ameko E, Achio S, Alhassan S, Kassim A. Microbial safety of raw mixed-vegetable salad sold in Ghana. J Med Biol Sci 2012; 1:1-8. doi: 10.5897/AJB11.2604
3. Beatrice O, Oguntoyinbo T. Production of tofu from blends of soybean and sesame seed. Afr J Food Sci 2012; 6:386–391. doi: 10.5897/AJFS12.043
4. Bristone C, Mshelia HA, Ogori FA, Badau MH, Ogori J. Microbial quality evaluation of awara (Soybean cheese) processed and sold at university of maiduguri campus. J Bacteriol Mycol Open Access 2018; 6:30–34. doi: 10.15406/jbmoa.2018.06.00172
5. Costanzo N, Ceniti C, Santoro A, Clausi MT, Casalinuovo F. Foodborne pathogen assessment in raw milk cheeses. Int J Food Sci 2020; 5. doi: 10.1155/2020/3616713
6. Egbo AO, Seidu KT. Microbiological evaluation of suya (diel smoked meat) sold in Ado and Akure, south west: Nigeria. Eur J Exp biol 2012; 1:1–5.
7. Elfaki AE, Elhakim SA. Quality evaluation of two Sudanese street foods of animal origin, Adv J Food Sci Techs 2011; 3:219-223.
8. Farah Z, Fischer A. Milk and meat from the camel handbook on products and processing 2004.
9. Fasoyiro SB. Assessment of hazards in local soy-cheese processing: Implication on health and environment in Oyo state, Nigeria. WIT Transaction on Ecoland Env 2011; 152:37-44. doi: 10.2495/FENV110041
10. Fukuda K. Food safety in a globalized world. Bulletin of world health organization 2015; 93:212. doi: 10.2471/BLT.15.154831
11. Hunter BB, Bamett HL. Deuteromycetes (fungi imperfection). In handbook of microbiology, Laskin AI, Lechevalier HA (Eds) 4th edition, CRC Press, Boca Raton FL 2000; 1-234.
12. Ibrahim TA, Falegan CR. Antibacterial activities of crude cell free supernatants of lactic acid bacteria from wara (Nigeria

soft cheese). Research and reviews. J Food and Dairy Technol 2013; 1:1-4.
13. Kolawole OF, Opeolu MO, Adenike OO, Olanrewaju E. Physio-chemical, sensory and microbiological characteristics of plain yoghurt from Bambara groundnut (*Vigna subterranean*) and soybeans (*Glycine max*). J Food Sci Technol 2015; 52:5858-5865. doi: 10.1007/s13197-014-1657-3
14. LaBarbera M. List of Types of Cheeses 2012. www.nourish interactive.com accessed 2019.
15. McDonald Scott Chinese top food safety official resigns 2018. NBCNEWS. https://en.m.wikipedia.org.
16. Muhammad RH, Yaro CA, Balarabe MB. Zainabja JA, Adedayo MR. Assessment of bacteria associated with ready-to-eat food sold at Federal University Dutse, Jigawa State, Nigeria. Int J Curr Res Bio sci Plant Biol 2016; 3:5-14. doi: 10.20546/ijcrbp.2016.304.002
17. Nazim MU, Mitra K, Rahman MM, Abdullah ATM, Parveen S. Evaluation of the nutritional quality and microbiological analysis of newly developed soya cheese. J Inter Food Res 2013; 20:3373-3380.
18. Ogbolu DO, Terry AAO, Oluremi AS, Olanrewaj AA. Microbial contamination of locally produced cheese and determination of their antimicrobial potential in Nigeria. Afri J Clin Exp Microbio 2014; 15:76-83. doi: 10.4314/ajcem.v15i2.4
19. Owusu-Kwarteng J, Akabanda F, Dominic-Agyei D, Jespersen L. Microbial safety of milk production and fermented dairy products in Africa 2020; 752. doi: 10.3390/microorganisms8050752 Microorganisms
20. Oyeleke SB, Manga SB. Essentials of laboratory practical in microbiology. To best Publisher, Minna, Nigeria 2008; 36-75.
21. Rawat S. Food spoilage: Microorganisms and their prevention. Asian J Plant Sci Res 2015; 5:47-56. doi: 10.12691/ajfn-4-5-2
22. Saleh I, Al-Thani R. Fungal food spoilage of supermarkets' displayed fruits, Vet World 2019; 12:1877-1883. doi: 10.12691/ajfn-4-5-2
23. Schaeffer J. Dairy food substitutes - the sky's the limit 2012. www.todaysdietitian.com/newsarchives/080112p38.shtm
24. Tamime AY, Muir DD, Wszolek M, Domagala J, Metzger L, Kneifel W, Dürrschmid K, Domig KJ, Hill A, Smith A, Guinee TP, Auty MAE. Quality control in processed cheese manufacture and analogues 2011; 245-340. doi: 10.1002/9781444341850.ch10
25. Tauxe RV. Emerging foodborne pathogens. Int J Food Microbiol 2002; 78:31-41. doi: 10.1016/S0168-1605(02)00232-5
26. Willey JM, Sherwood LM, Woolverton CJ. Prescott Microbiology. 8th ed. McGraw-Hill Companies, New York 2011; 1070.
27. World Health Organization (WHO) estimates global burden of foodborne diseases Foodborne diseases burden epidemiology 2015. http://www.who.int/foodsafety/publications/foodborne_disease/fergreport/en/
28. World Health Organization (WHO) programmed on the global burden of food borne disease: Fact Sheet: Key Facts on Food Safety 2020. http://www.who.int/foodsafety/publications/foodborne_disease/fergreport/en/
29. Yusuf HL, Ali AI. Production, proximate and sensory assessment of awara produced from fresh and sun-dried soybean curd mix. Bayero J of Pure Appl Sci 2013; 6:100-103.